Air-water CO_2 fluxes in a highly heterotrophic estuary.

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1. Introduction

Estuaries, due to their transition location, are affected by both marine processes, such as tides, waves, intrusion of saline water, and riverine processes like flows of freshwater and sediments. They also represent an important source of nutrients to the coastal area, as estuarine systems are considered regions of a high productivity worldwide (Mann 1982). Nutrient inputs in estuaries (particularly nitrate) are affected by water discharge, entries from both natural (groundwater transport, riverine inflow and atmospheric deposition) and anthropogenic sources (sewage treatment plants, agricultural and lawn fertilizers etc), and resident estuarine processes, such as denitrification and nitrification (Soetaert et al. 2006).

Elevated nutrients concentrations spur rapid phytoplankton growth and the occurrence of periodic microalgal blooms that eventually lead to the generation of considerable organic loads. Subsequent bacterial decomposition of this organic matter promotes oxygen depletion and thus hypoxic or anoxic zones may commonly appear in estuaries (Conley et al., 2009). The genesis of hypoxia can be traced by specific nitrogen compounds, as nitrification processes decrease oxygen concentration, and denitrification principally proceeds in suboxic conditions. Hypoxia in estuaries represents a common disturbance (Cox et al., 2009; Sharp, 2010) causing death of biota and catastrophic changes at the entire ecosystem level (Vaquer-Sunyer and Duarte, 2008). Not only organisms and their habitat result affected by hypoxia, but also the biogeochemical processes that control nutrient concentrations in the water column (Conley et al., 2009). Moreover, turbulence induced by the meeting of the downstream freshwater flow and the upstream tidal flow, and flocculation of material induced by the mixing of fresh and salt water, increase residence times of suspended particulate matter (SPM), forming maximum turbidity zones (MTZ), which are generally considered the most important regions in a tidally influenced estuary, where photosynthesis becomes strongly limited by light availability (Abril et al., 1999; Amann et al., 2012; Etcheber et al., 2007; Garnier et al., 2010; Herman and Heip, 1999). Heterotrophic activity is in turn enhanced ought to the high turbidity, resulting in a net mineralization of a major part of the particulate organic carbon (POC) (Gattuso et al., 1998). As a final consequence, high levels of CO_2 generated by degradation of dissolved and/or particulate organic carbon and by other anaerobic processes in addition to denitrification, such as sulphate reduction and in some anaerobic environments methanogenesis (Richey et al., 1988), are released to the atmosphere. This kind of estuaries act then as significant sources of CO_2 to the atmosphere due to their net heterotrophic metabolic status (Frankignoulle et al., 1998; Chen and Borges, 2009; Laruelle et al., 2010; Borges and Abril, 2011; Chen et al., 2012; Chen et al., 2013; Testa et al., 2013).

Given the complexity of the biogeochemical processes involved in the heterotrophy of estuaries, integrated studies performed at a high spatio-temporal scale are needed to properly characterize the dynamics of these systems. Furthermore, in order to determine the contribution of estuaries to the CO_2 emissions and the horizontal fluxes of carbon, high resolution data with a proper degree of accuracy are required to obtain and provide precise estimates of the air-water CO_2 exchange in these environments.

Within this context, this work was aimed at examining the temporal and spatial variability of the carbon system parameters in the tidally dominated Guadalquivir estuary (SW Spain, Fig.1) in relation to the metabolic status of the system. Despite its socio-economic and environmental significance (Ruiz et al., 2009), until very recently only one study addressing the characterization of the inorganic carbon system inside the estuary had been published (de la Paz et al., 2007). On other hand, several works have been carried out in the adjacent coastal fringe of the Gulf of Cádiz, where the Guadalquivir river discharges (Huertas et al., 2006; Ribas-Ribas et al., 2013) or in the wetlands of Doñana National Park, located in the northern rim of the lower estuary (Morris et al., 2013). The Guadalquivir estuary is characterized by a strong turbidity (Navarro et al., 2011; Caballero et al., 2013), which markedly restricts light availability for phytoplankton growth (Navarro et al., 2012). Light limitation seems to be a constant feature within the estuary, thereby constraining primary productivity even though high nutrients loads are present in the water column (Navarro et al., 2012, Ruiz et al., 2014). The seasonal shift in hydrology from winter river-dominated (rainfall season) to tidally dominated flow is the main factor controlling the patterns of nutrients and suspended matter in the estuary (Diez-Minguito et al., 2012), as during the river-dominated period considerable freshwater inputs from an upstream reservoir (the Alcala del Rio dam) are introduced in the system. Due to the relevance of the estuary on its adjacent areas, we assessed the spatio-temporal distribution of carbon system parameters in the system during nearly two years of sampling in relation to biogeochemical variables indicative of heterotrophy. The analysis was ultimately aimed at determining the air-water CO_2 exchange in the estuary and the processes governing gas transference.

2. Material and methods

2.1. Study area

The Guadalquivir River, located in Southern Spain (Fig.1), is one of the longest rivers in the Iberian Peninsula, with a total length of 560 Km and a drainage area of 57,527 km^2. The estuarine region hosts 1.7 million inhabitants, which are distributed on 90 population settlements (Navarro et al., 2012). Due to the socio-economic importance of the estuary (agriculture, fisheries, tourism, etc.), it is affected by an intense anthropogenic pressure. Guadalquivir is the only large navigable river in Spain, reaching to the port of Sevilla. Therefore, maintenance activities of the navigation channel take part regularly in the estuary, including locks building and dredging works to deepen the main channel, in order to allow big ships the access into Seville'sport (Ruiz et al., 2014).

The estuary extends to the Alcala del Rio dam, which is situated 110 km upstream from the river mouth, being characterized by a main channel surrounded by tidal creeks with a few significant little intertidal zones. As a mesotidal system, the tidal influence is noticeable up to the dam and the maximum tidal range at river mouth is 3.5 m during spring tides (Díez-Minguito et al., 2012).

The basic climate in the region is Mediterranean, defined by warm temperatures (16.8°C annual average) and with relatively short periods of heavy rainfall occurring during winter (annual average of 550 l m^{-2}). The Atlantic Ocean orientation allows storms entering from West, driving the distribution of rainfall SW-NE. The rainfall often adopts torrential character acting on a system recurrently affected by long dry periods, high temperatures and consequently with a marked erosion susceptibility (Memoria de la Confederación Hidrográfica del Guadalquivir 2009/2011, Ministerio de Agricultura, Alimentacion y Medioambiente, Gobierno de España).

2.2 Sampling strategy

Monthly samplings were carried out at 12 stations between November 2007 and August 2009 (Fig.1). From station 1 positioned at the river mouth, stations 2 to 12 were located at 6, 16, 22, 25, 34, 46, 55, 63, 71, 78 and 82 Km upstream, respectively. At each station, a CTD (Conductivity, Temperature and Depth), salinity and turbidity profiles were obtained with a SeaBird SBE 19 plus equipped with a Turbidimeter Cyclops-7sensor (Turner Designs), which was followed by collection of water samples at 1 m depth with a Van Dorn Bottle Sampler for determination of the following biogeochemical variables: Total alkalinity

(A_T), pH, dissolved oxygen (DO), suspended particulate matter (SPM), chlorophyll (Chl*a*), inorganic nutrients (NH_4^+, NO_2^-, NO_3^-, PO_4^{-3} and Si), dissolved organic carbon (DOC) and total dissolved nitrogen (TDN).

2.3 Measurements protocols

Water samples for A_T determination were collected in 500 ml borosilicate bottles and poisoned with 100 µl of $HgCl_2$ saturated aqueous solution until analysis in the laboratory according to the DOE handbook protocols (Dickson and Goyet, 1994).A_T was measured by potentiometric titration with a Metrohm 794 Titroprocessor using the method described by Mintrop et al. (1999). The precision and accuracy of A_T determinations were between ±5.3 and 2.1 µmolkg^{-1}respectively, as determined from daily measurements of 2 batches (#94 and #97, n=44) of certified reference material (CRM, supplied by Prof. Andrew Dickson, Scripps Institution of Oceanography, La Jolla, CA, USA). Constants used for A_T calculations as for boric acid and $KHSO_4$ were those from Cai and Wang (1998), Lee et al. (2010) and Dickson (1990b), respectively. Water pH measurements in NBS (National Bureau of Standards) scale (pH_{NBS}) were carried out from the A_T samples using a Metrohm 780 pH meter equipped with a combined glass electrode. Calibrations were conducted daily using NBS buffers at three different pH values, 4, 7 and 9. Precision and accuracy of the pH measurements were 0.001 and ±0.003 pH units, respectively.

Dissolved oxygen was measured using an automated potentiometric modification of the original Winkler method following WOCE standards (WOCE, 1994.). Flasks containing the water samples were sealed, stored in darkness and measured within 24h. Dissolved oxygen concentrations were derived using a Metrohm 794 Titroprocessor, with an estimated error of ± 2 µmol l^{-1} (n=115).

SPM as well as particulate organic matter (POM) and particulate inorganic matter (PIM) were determined by using the Loss On Ignition (LOI) method. A known volume of water was filtered through pre-combusted 450º C Whatman GF/F glass fiber (0.7 mm pore size). Filters were dried at 60ºC for 48 h and weighed to derive SPM (g l^{-1}), further combusted at 450ºC for 5h and weighed to derive PIM and POM by difference.

Chlorophyll analysis was conducted by filtering known volumes of water through Whatman GF/F glass fiber filters, extracting in 90 % acetone overnight in the dark, and measuring chlorophyll a concentrations

(Chl*a*) by fluorometry with a Turner Designs 10-AU Model fluorometer according to the JGOFS protocol (Knap et al., 1996b) . The fluorometer was calibrated using a pure Chl*a* standard from the cyanobacterium *Anacystis nidulans* (Sigma Chemical Company).

Inorganic nutrients sampling was performed following the methodology described by Knap et al. (1996a). Two 5 ml samples of filtered water (Whatman GF/F glass fiber) were obtained and stored at -20ºC until analysis. Concentrations of NH_4^+, NO_2^-, NO_3^-, PO_4^{-3} and Si were derived following the techniques described by Grasshoff et al. (1983) using a Skalar San++ 215 Continuous Flow Analyzer. For the analysis of DOC and TDN, water samples were collected in borosilicate vials (pre-acid-washed and-combusted, 450ºC). Water was filtered with pre-combusted 450ºC Whatman GF/F glass fiber 47 mm filters and 24 ml sub-samples were acidified with 50 µl of 25% H_3PO_4, sealed and conserved at 4ºC in darkness until analysis. Samples for DOC and TDN were obtained according a modification of the JGOFS protocol (Knap et al., 1996a). Concentrations of DOC and TDN were derived by catalytic oxidation at high temperature (720ºC) and chemiluminescence, respectively using a Shimadzu TOC-VCPH analyser TDN was subsequently used to obtain Dissolved organic Nitrogen (DON) concentrations by subtracting inorganic nitrogen (Álvarez-Salgado and Miller, 1998).

The diffuse attenuation coefficient for photosyntetically active radiation (PAR) was calculated by the following expression obtained from Gallegos (2001):

$$K_d = K_w + K_c\,[Chl a] + K_s\,[SPM] \quad (1)$$

where K_W (0.26 m^{-1}) is the attenuation due to water alone, and K_C (0.017, (m^2 (mg Chl)$^{-1}$) and K_S (0.074 m^2 gr^{-1}), stand for the specific-attenuation coefficient of chlorophyll and SPM, respectively. The diffuse attenuation coefficient (K_d) was used to estimate the lower limit of the euphotic zone ($Z_{1\%}$) defined as the depth where the PAR represents 1% of the surface radiation, following the Lambert-Beer law (Kirk, 1994):

$$Z_{1\%} = Ln\ (0.01) / K_d \quad (2)$$

Calculations of air-water CO_2 fluxes

For fluxes calculations, $[pCO_2]_{water}$ and dissolved inorganic carbon (DIC) were obtained from A_T and pH_{NBS} pairs by using the CO2SYS software(Lewis et al., 1998) with the thermodynamic equations and constants for carbon and sulphate of Cai and Wang (1998) and Dickson (1990) respectively.

Air-water CO_2 fluxes (FCO_2 in mmolCO_2 m^{-2} d^{-1}) were calculated according to Wanninkhof et al. (2009):

$$FCO_2 = k_w\, k_0\, ([pCO_2]_{water} - [pCO_2]_{air}) \quad (3)$$

where, k_w (m s^{-1}) is the water–side gas transfer velocity, k_0 (mol m^{-3} atm^{-1}) is the aqueous-phase solubility of CO_2 at the *in situ* temperature (ºC) and salinity (Weiss 1974; Wanninkhof 1992), and $[pCO_2]_{air}$ in µatm is the atmospheric pCO_2 values obtained from the NOAA Lampedusa (Italy) monitoring station (http://www.esrl.noaa.gov/gmd/dv/site/) and then converted from pCO_2dry to wet (Weiss and Price, 1980). k_w was calculated using:

$$k_w = k_{600}\, (S_{cw} /600)^{-0.5} \quad (4)$$

where, S_{cw} is the Schmidt number interpolated at the *in situ* salinity from the freshwater and sea water equations (see Wanninkhof, 1992). k_{600} is the gas transfer velocity at a Schmidt number of 600 (often quoted as typical of freshwater at 20ºC). k_{600} was predicted from time–ensemble averaged (1 d) horizontal wind velocity at 10 m above the surface (U_{10}m s^{-1}) using the empirical relationships of Raymond and Cole (2001),

$$k_{600} =1.58\, e^{0.3}\, U_{10} \quad (5)$$

where k_{600} is in cm h^{-1}. U_{10} was calculated from U_z measured at nearby meteorological stations (Estaciones Agroclimaticas, Junta de Andalucia, Spain; Fig. 1) according to Smith (1988). For comparison, k_{600} proposed by Clark et al. (1995) and the recently revised by Jiang et al. (2008) were also used.

Daily discharge data from the Alcala del Rio dam and precipitation from the Lebrija Brazo del Este Station (Precipitation station in Fig.1) were obtained from the Sistema Automático de Información Hidrológica of the Guadalquivir basin (http://www.chguadalquivir.es/saih/Inicio.aspx).

Statistics

Statistics were performed with the statistical program language R 3.02 (R Development Core Team, 2013). Probability distributions of variables were examined visually and in most cases were log-normal and highly skewed. Non-parametrical Kruskal-Wallis rank sum tests (KWRS, R function; kruskal.test) and non-parametrical multiple test procedures (KWMC, Package; pgirmess, function; kruskalmc) were used to examine differences between zones (Giradoux, 2012). Significance levels were set at $p<0.05$. Principle components analysis (R package; FactoMineR, function; PCA, Husson et al., 2012) of transformed, log(x+1), variables, with monthly mean wind speed, monthly river discharge and surface temperature as a supplementary quantitative variables, was used to explore correlations. Linear regressions between variables were also used to establish linear relationships.

3. Results and discussion

Estuary partitioning: spatio-temporal distribution of thermohaline properties

PCA revealed that data could be summarised into 5 components that accounted for a cumulative percentage variance of 77% (Fig. 2), with values from PC1 to PC5 of 30.73, 19, 11.54, 8.75 and 6.63%, respectively. In terms of briefly characterising, only principle components 1 (PC1, Dim 1) and 2 (PC2, Dim 2) are discussed here. PC1 appears to represent spatial distribution and thus led us to divide the estuary in 3 regions. Positive PC1 values mainly represented higher values of dissolved material (A_T, DOC, and DON) corresponding to stations 9 to 12 (Fig. 1), which then defined the Inner Estuary (IE). Negative PC1 appears to represent elevated values of salinity, DO and pH corresponding to stations 1 and 2 (Fig. 1), characterizing hence the Lower Estuary (LE). The transitional zone between the inner and lower estuary, comprising stations 3 to 8 (Fig. 1), was then designed as the Middle Estuary (ME). IE was characterized by the presence of the lowest dissolved oxygen concentrations (Fig. 3) representing thus the Oxygen Minimum Zone (OMZ). During our study, oxygen levels were as low as 1.1 mg l^{-1} (Table 1), suggesting that OMZ can, at times, be classified as severely hypoxic. As defined by the Environmental Protection Agency (EPA, 2000), hypoxia in aquatic ecosystems occurs at oxygen levels below 2.9 mg l^{-1}, with severe hypoxia being marked by concentrations <2.3 mg l^{-1}. In contrast, oxygen levels in the ME and LE were ~ 6 mg l^{-1}in both zones.

Salinity range in the IE was mesohaline, with salinity values < 5 (Fig. 3 and Table 1), which progressively increased throughout the estuary to finally reach 36 in the river mouth (LE, Fig. 3). The spatial distribution of salinity within the entire estuary may be attributed to the combination between the tidal flooding and variability in river discharge from the dam (Navarro et al., 2012, Díez-Minguito et al., 2013).

In addition to salinity linked to PC1, turbidity was also selected as a variable defining spatial variability, due to its relevance on the ecosystem dynamics (Navarro et al., 2012, Ruiz et al., 2014). Turbidity maximum was located within the ME, defining the Maximum Turbidity Zone (MTZ) (Fig. 3 and Table 1).

On the other hand, PC2 seems to describe seasonal variations during the study period, as negative values represent high temperature and Chl*a*, both mostly appearing during spring-summer (Fig 2). Conversely, positive values define autumn-winter conditions with increased precipitations and particulate matter (PM) contents. The highest spatial variability was found invariably in the ME, whereas at a temporal scale spring season seemed to be the most fluctuating period for the whole estuary (Fig. 2).

Surface water temperature pattern was typical of the Mediterranean climate area, with hot summers and moderate cold winters. During the study period, water temperature values ranged from 4.6 to 27.7ºC (Table 1), with no statistical differences between zones (KWRSχ^2= 2.2, df (degrees of freedom) =2, *p*-value > 0.05).In contrast, neither salinity nor dissolved oxygen values presented any seasonal variation (KWRS *p*-value > 0.05).

Chlorophyll, turbidity, water discharge and light penetration

Guadalquivir estuary can be categorized as one of the most turbid rivers in the world, with average SPM values of 650.5 mg l^{-1}(Table 1). For instance, rivers such as Huanghe (China), Mackenzie River (Canada), Amazon (Brazil) and the Scheldt estuary (Belgium) have mean SPM concentrations of 23, 830, 320, 97 and 100 mg l^{-1} (Gaillardet et al., 1999; Kim et al., 2010; Chen et al., 2005), respectively. This is also shown by the turbidity values provided by the CTD sensor (Table 1), which markedly varied along the different zones of the estuary (KWRS, χ^2 = 63.1, df= 2, *p*-value < 0.001). The high turbidity in the

Guadalquivir estuary can be related to the generally long residence time and with the occurrence of high mesotidal currents (Díez-Minguito et al., 2012; 2013).

As illustrated in Figure 4B, K_d values manifestly changed throughout the year (KWRS χ^2= 39.1, df=3, *p*-value < 0.001), with a maximum of 400 m^{-1} reached in spring 2009 and a minimum of 1.5 m^{-1} in autumn 2008, resulting in changes in the euphotic depth from -0.01 to -3.1 m^{-1} (Fig. 4 C) . Maximum K_d values where generally observed during periods of high precipitation or high discharges from the Alcala del Rio dam (Fig. 4 A, B).When high loadings occurred, light penetration was more limited and hence the lower limit of the euphotic zone shallowed (Fig.4B). As a result, primary production was expected to be highly restricted due to reduced light availability and as seen also in Taglialatela et al. (2014) zooplankton dominant species decreased to extremely low values during this events.

Chl*a* appeared to exhibit a seasonal trend (Fig. 4 D), with spring blooms in 2008 and 2009 with maxima of 10µg l^{-1} and the lowest concentrations around 0.1 µg l^{-1}during autumn and winter coinciding with increased precipitations and discharges. Nevertheless, Chl*a* concentrations were lower than the expected phytoplankton biomass that could be supported by the nutrient levels present in the estuary (Ruiz et al., 2014), feature that can be attributed to light limitation, as shown by the K_d and $Z_{1\%}$values, which indicate that light penetrated on average only 5% into the water column.

Carbon system parameters

Total Alkalinity, plotted in Figure 5, was characterized by a marked spatial variability (KWRS χ^2= 83.5, df=2, *p*-value <0.001), with the highest values measured being associated with the transition between IE and ME (KWMC p<0.05). Minimum A_T concentrations were found in the LE, as the proximity to the coastal area allows the inflow of seawater characterized by a lower A_T. In fact, in this part of the estuary, the mean alkalinity during the whole study period was 2632.52±272.82 µmol kg^{-1}(Table 1), similar to that measured in the continental shelf of the Gulf of Cádiz and equivalent to 2402.5±8 and 2360±2 (Ait-Ameur and Goyet, 2006; Flecha et al., 2012). In contrast, waters with higher alkalinity were found upstream, with average values of 3468.18±403.15 and 3505.33±327.98 µmolkg^{-1} in the ME and IE, respectively. As reported previously by de la Paz et al. (2007), this alkalinity pattern can be partially related to the carbonate materials presented in the drainage basin of the upper part of the estuary (Pérez-

Hidalgo and Trinidad, 2004) and the mixing between fresh and marine waters. A_T values did not present any seasonal variation during the study period (Table 2).

The distribution of pH_{NBS} values also exhibited a strong spatial (KWRS χ^2= 95, df=2, *p*-value <0.001) and temporal variability (KWRS χ^2= 48.6, df=2, *p*-value <0.001). No statistical differences were observed (KWMC p<0.05) between spring and autumn whereas significant differences were found among the rest of seasons, with winter being the period in which lower pH_{NBS} values were measured (Table 2). The spatial variability of pH_{NBS} in the IE (Table 1, Fig. 5b) can be associated to the freshwater discharges from the dam. Conversely, higher pH_{NBS} values found in the LE may reflect the entry of saltier and oxygenated waters as well the influence of the DIC fluxes from the river. In opposition, pCO_2 exhibited a gradual increasing gradient upstream (Fig. 5C), as seen in other estuaries (Chen et al., 2012) and already reported in this system (de la Paz et al., 2007). Extremely high pCO_2 concentrations (between 4000-5000 µatm) were detected in the IE, where pCO_2 levels always exceeded 1000 µatm during the whole study period (Fig. 5c). In the LE, average values also coincide with those measured in previous studies in the area (de la Paz et al., 2007; Ribas-Ribas et al., 2011), presenting during certain short periods, pCO_2 values below the atmospheric concentration (Table 1). Nevertheless, our data show that the Guadalquivir estuary, in average, was constantly characterized by pCO_2 values higher than the atmospheric concentration. These CO_2 concentrations are higher in relation to those observed in the past, which fell within a range from 518 to 3606 µatm from low to high salinity (de la Paz et al., 2007).

Taking salinity as a good indicator for mixing of dissolved compounds subject exclusively to physical processes, a linear relationship between DIC and salinity can be obtained, which allows to estimate the concentration of inorganic carbon in freshwater. Our calculations (DIC=3.68-0.034*S, r^2=0.64, n=248) yield then a value of carbon in freshwater corresponding to 3.68 mmol kg^{-1} and considering a historic average discharge of 160 $m^3\ s^{-1}$ (Ruiz, 2010), the transport of inorganic carbon by advective processes from the Guadalquivir estuary to the continental shelf of the Gulf of Cádiz resulted in 50 x 10^6 mol C d^{-1}, indicating that the system has the potential to channelize an advective export of approximately 219,000 tons of C per year.

Air-water CO_2 exchange

Fluxes of CO_2 between the atmosphere and the water column (FCO_2) throughout the Guadalquivir estuary were calculated using three gas transfer velocities (k_{600}) for comparison. No large differences were detected between the outputs obtained with the parameterizations of Clark et al. (1995) and Raymond and Cole (2001), as k_{600} ranged from 2.54 to 7.26 and from 2.13 to 6.69 cm h^{-1}, respectively. In contrast, when the Jiang et al. (2008) parameterization was used, an increase in k_{600} was observed for the whole wind range (from 3.89 to 8.98 cm h^{-1}), as this formulation magnifies the coefficient values for wind speeds below 5 m s^{-1}. Taking into account this caveat and in order to compare with historic data, only fluxes calculated according to Raymond and Cole (2001) were subsequently considered in our study (Fig. 6).

The temporal distribution of FCO_2 indicated that estuarine waters were oversaturated with respect to the atmosphere during most of the study period (Fig. 6), with average annual FCO_2 values decreasing from 66.9±18.6 to 29.4±20.3 and to 3.4±8.1 mmol C m-^{2} d^{-1} in the IE, ME and LE, respectively (Table 3). Accordingly, the IE behaved as a strong CO_2 source to the atmosphere whereas the LE captured CO_2 during certain periods, particularly during the spring and summer months (Fig. 6). Overall, higher fluxes coincided with water discharges from the dam and increase in rainfall (Fig. 4A), although noticeable exchange variability was invariable found in the ME (Fig. 6). Nevertheless, CO_2 seasonal and annual fluxes were in the same order of magnitude than those measured in other estuarine areas and fall within the range compiled by Chen et al. (2013). In the Guadalquivir estuary in particular, the annual CO_2 flux from 2000 to 2003 calculated by using summer measurements along different sites of the estuary included now in the LE and ME of our division was 37.9 mol C m-^{2}yr^{-1} (de la Paz et al., 2007). This value matches quite well that of 36.4±11.7 mol C m-^{2}yr^{-1} obtained in our study for the whole system (Table 3). However, these authors also reported higher instant fluxes (around 114.3-128 mmol C m^{-2} d^{-1}) even though lower pCO_{2water} levels than those found here (Table 2) were present. This discrepancy may be mainly attributed to the average wind speeds, which were manifestly higher in the prior study (7.35 m s^{-1}) as compared to those measured during our sampling period (1.96 m s^{-1}), with less significance on the different k_{600} parameterizations used in both studies. Recently, Morris et al. (2013) provided FCO_2 at the adjacent Doñana wetlands fed by the Guadalquivir river that ranged from -4.1 to 5.5 mmol C m-^{2} d^{-1} and with a mean U_{10}value of 2.5 m s^{-1}, which are in the same order of magnitude than those computed at the LE adjacent to the Park (Fig. 6c and Table 3).

The pattern of both pCO_2 and air-water CO_2 exchange suggests that heterotrophy controls the metabolic status of the estuarine waters. Thus, the strong correlation found between pCO_2 and the Apparent Oxygen Utilization (AOU) (R^2=0.800, *p*-valor<0.001, Fig. 7) indicates that pCO_2 levels were mainly controlled by biological processes, especially organic matter degradation. In the estuary, the organic carbon component was significant, as indicated by the high DOC concentrations measured, with values varying from 1.5 to 14.5 mg l^{-1} (Table 1). No seasonality was found in the DOC levels (KWRS χ^2= 95, df=3, *p*-value <0.05) although their spatial distribution revealed a clear variability (KWRS χ^2=31.6, df=2, *p*-value <0.001). This finding reflects the effect of mixing as higher DOC concentrations were present upstream in fresh water (Fig. 8) and a more dispersed pattern, with no statistical differences as in the case of turbidity, in the IE and ME (KWMC *p*<0.05;Table 1). Using the criteria described by Abril et al. (2002), according to the average DOC concentration (5.98±2.24 mgl^{-1}, *n*=184), and the considerable anthropogenic pressure over the system (Ruiz et al., 2014), the Guadalquivir estuary can be categorized as heterotrophic and its metabolic status is comparable to that of the Sado and Loire rivers. Another piece of evidence indicative of the heterotrophic status is the inorganic nutrients levels present during the study period (Fig. 9), which were also significantly different for each estuary zone (KWSR *p*<0.001). The highest concentrations of nitrate, nitrite, ammonium and silicate were found in the IE and ME, with average values around 300, 6, 19 and 100 µM respectively (Table 1, Fig. 9), with a continue decrease towards the river mouth being observed (Fig. 9). On the other hand, phosphate showed a more homogeneous spatial distribution (Fig. 9) and average concentrations did not exceed 3 µM (Table 1). Seasonally, only nitrite and phosphate did not present significant variations (KWRS *p*>0.05). Mendiguchía et al. (2007) reported lower nutrient content in the system, particularly for NO_3^-, although sampling was performed twice a year and with river discharges from the dam substantially lower than those observed during our study period.

The stoichiometric ratio N:P evidenced an excess of nitrogen in the system, as values were comprised within the range of 173-1000, which is, on average, 12 times higher than the optimum Redfield ratio for phytoplankton growth (Redfield 1934). The N:P ratios found here are of the same order of magnitude than that observed in the Pearl River estuary and, in general, characteristic of systems that receive inputs from inland waters enriched in nitrogen relative to phosphorus (Harrison et al., 2008).

In addition, other processes such as denitrification, could partially contribute to the nutrient content in the estuary, particularly because denitrification is favoured in heavy turbid and nitrogen enriched waters, even under oxic conditions (Liu et al., 2013). From ammonia and nitrate reduction, the nitrification-denitrification process results in increased concentrations of the intermediate NO_2^-component under oxic and anoxic conditions, respectively. Consequently, a rise in N_2O emissions to the atmosphere is commonly observed. In the Guadalquivir estuary, concentrations of NO_3^-,NO_2^- and NH_4^+ in the ME (MTZ) and the IE (OMZ), were significantly different from those in the LE (KWMC p<0.05) and substantially higher (Table 1 and Fig. 9), which may be indeed indicative of an enhanced denitrifying microbes activity. In addition, pH_{NBS} values in the IE (7.80±0.17, Table 1) would favour denitrification (Soetaert et al., 2007) and the elevated A_T measured in this zone (Table 1) would also be indicative of the presence of denitrification, as seen in other anoxic estuaries (Abril and Frankignoulle, 2001). Considering that Ferrón et al. (2010) defined the coastal fringe connected to the Guadalquivir river mouth as a N_2O source to the atmosphere and the highest nitrous oxide saturation concentrations were found at the lowest salinity zone, denitrification may be well claimed as a relevant mechanism participating in the nitrogen balance of the estuary. Nevertheless, further denitrification measurements are required to support this conclusion.

4. Summary

Our work shows that the high turbid Guadalquivir estuary behaved as a net CO_2 source to the atmosphere during a nearly two year period (2007-2009) and a low net gas absorption was only detected in the more saline river mouth during short time intervals. Seasonal variations in the air-water CO_2 fluxes were generally well correlated with an increase in the discharge/rainfall regime in the area, although the permanent heterotrophy in the estuary was the main factor controlling the CO_2 levels in its waters. The very high CO_2 concentrations found in the system and comparable to other high heterotrophic and turbid estuaries were mainly due to the high organic matter and nutrient content (particularly nitrate), which favours respiratory decomposition and denitrification. This status was reflected in a pronounced hypoxia during certain periods and particularly in the inner zone of the estuary, which was also exacerbated by limitation in light penetration, hampering phytoplankton growth and the concomitant water oxygenation. Our data and comparisons with previous records reported in the area confirmed that the Guadalquivir estuary acts as a permanent potent CO_2 emitter to the atmosphere.

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Table 1: Average values, standard deviation and number of observations in brackets (n) of the variables presented in this study for the three areas defined in the Guadalquivir estuary.

Table 2: Average values, standard deviation and number of observations in brackets (n) of pH_{NBS} A_T (µmol kg^{-1}), DIC (µmol kg^{-1}) and pCO_2 (µatm) in each season.

Table 3: Spatio-temporal FCO_2 average values and standard deviation.

Figure 1: Location of the study area, sampling sites and meteorological stations. Position of the Alcala del Río dam is also shown.

Figure 2: Biplots of water physical and chemical characteristics with scaling highlighting variable correlations (A) and mapping of individual samples (B). Rainfall, wind velocity and Alcala del Rio dam discharge are plotted as supplementary variables (blue arrows and text) on A. Text representing the centroids for Lower Estuary (LE), Middle Estuary (ME) and Inner Estuary (IE) and seasons are shown on B.

Figure 3: Average values of salinity, dissolved oxygen (mg l^{-1}) and turbidity in Formazin Nephelometric Units (FNU) along the estuary, defining the three areas in which the estuary has been divided: LE, ME and IE.

Figure 4: Time distribution in the Guadalquivir estuary of: a) Daily averaged discharge (m^3 s^{-1}) in grey and precipitation (l m^{-2}) dotted red, b) Light extinction coefficient, K_d (m^{-1}) c) Euphotic zone, $Z_{1\%}$ (m^{-1}) and d) Chlorophyll *a*, Chl*a* (µg l^{-1}). Grey background bands highlight the periods with higher discharges in the estuary during the time of the study. The tops and bottoms of each "box" are the 25^{th} and 75^{th}

percentiles of the samples, respectively. The distances between the tops and bottoms are the interquartile ranges. The line in the middle of each box is the sample median. Observations beyond the whisker length are marked as outliers displayed with a black dot.

Figure 5: a) Total alkalinity (µmolkg^{-1}), b) pH (NBS scale), and c) pCO_2values (µatm) from the river mouth to 80 Km upstream. The tops and bottoms of each "box" are the 25th and 75th percentiles of the samples, respectively. The distances between the tops and bottoms are the interquartile ranges. The line in the middle of each box is the sample median. Observations beyond the whisker length are marked as outliers displayed with a black dot.

Figure 6: Spatio-temporal distribution of air-water CO_2 fluxes (FCO_2, mmol m^{-2} d^{-1}) in the Guadalquivir estuary during the study period for the: A) IE, B) ME and C) LE. Negative FCO_2values indicate that the area acts as a sink and positives as a source. The tops and bottoms of each "box" are the 25th and 75th percentiles of the samples, respectively. The distances between the tops and bottoms are the interquartile ranges. The line in the middle of each box is the sample median.

Figure 7: pCO_2 (µatm) values in relation to Apparent Oxygen Utilization (AOU) in the Guadalquivir estuary.

Figure 8: Dissolved organic carbon (mg l^{-1}) in relation to salinity in the Guadalquivir estuary.

Figure 9: Average concentrations (µM) of inorganic nutrients: nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), phosphate (PO_4^{3-}), and silicate (Si) from the river mouth to 80 upstream during the period of study. The tops and bottoms of each "box" are the 25th and 75th percentiles of the samples, respectively. The distances between the tops and bottoms are the interquartile ranges. The line in the middle of each

box represents the sample median. Observations beyond the whisker length are marked as outliers displayed with a black dot.

Figure1

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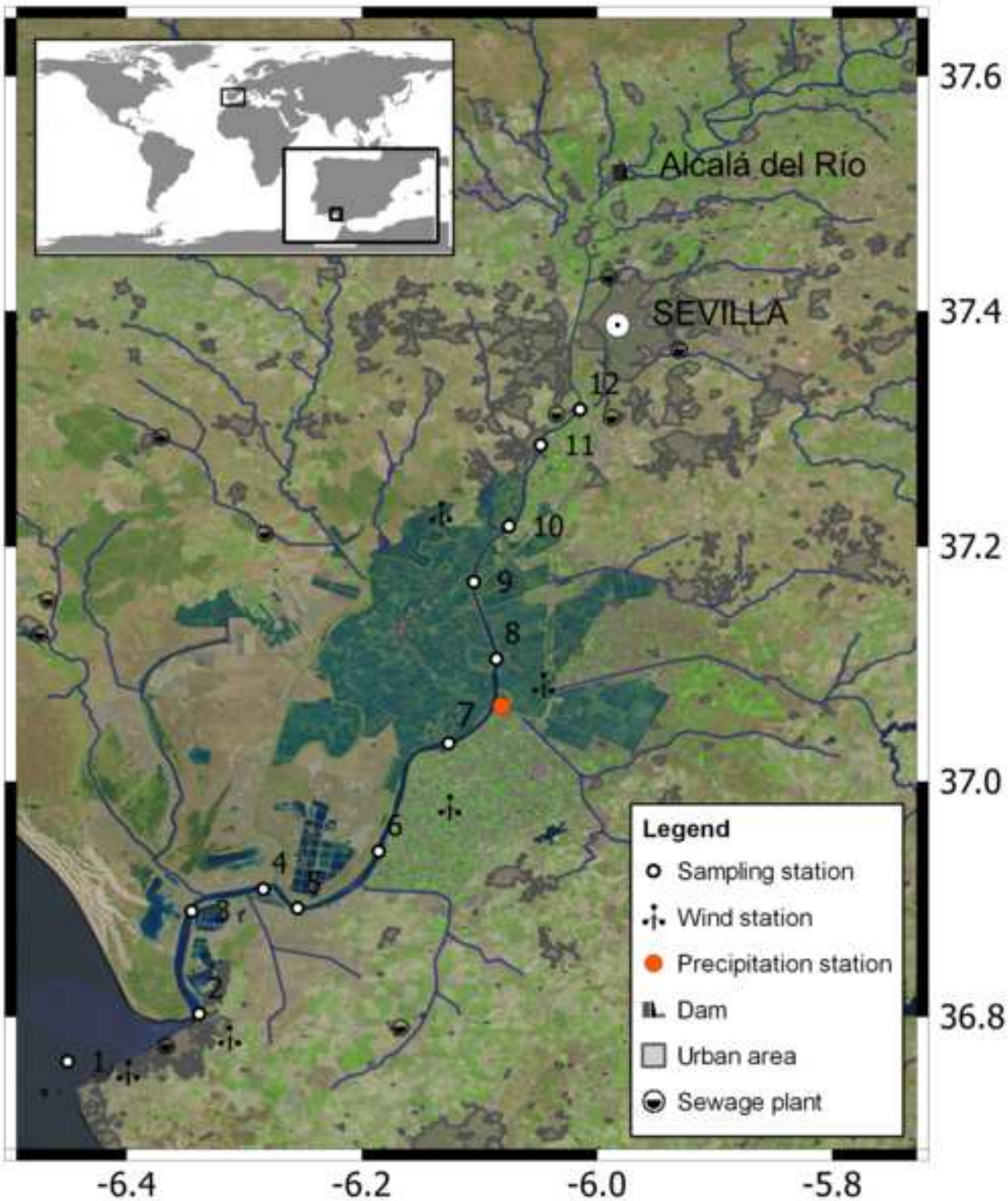

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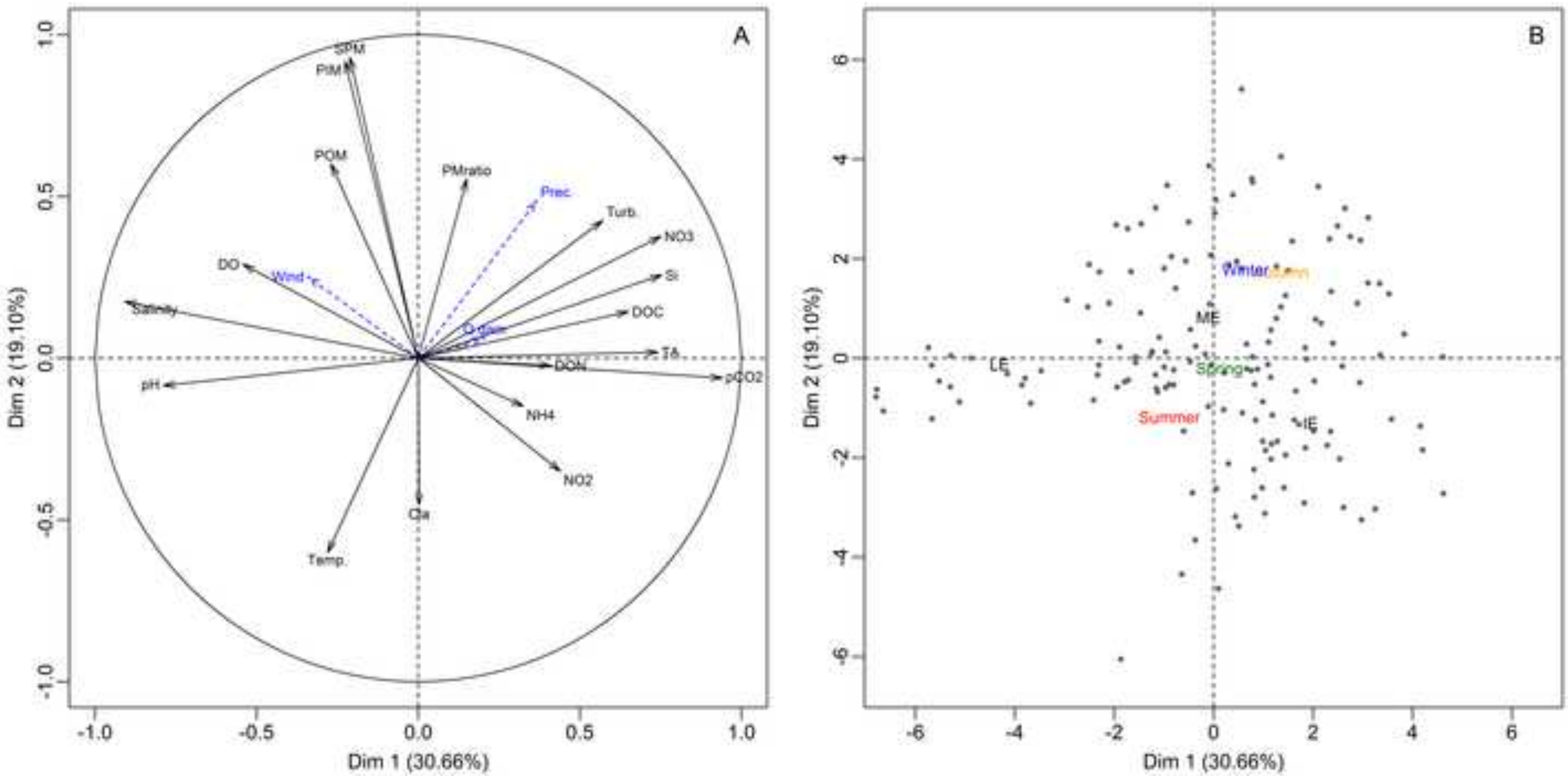

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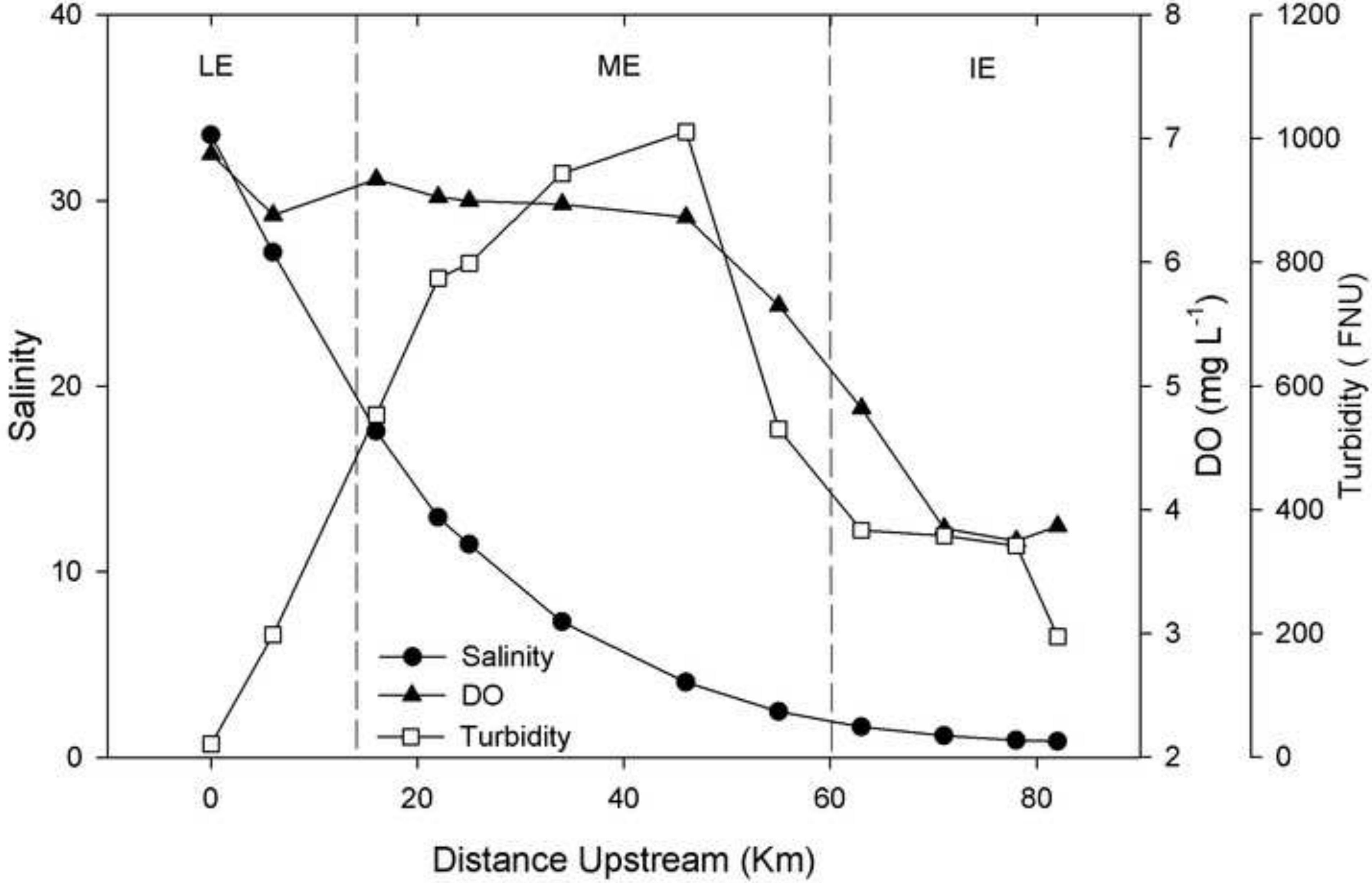

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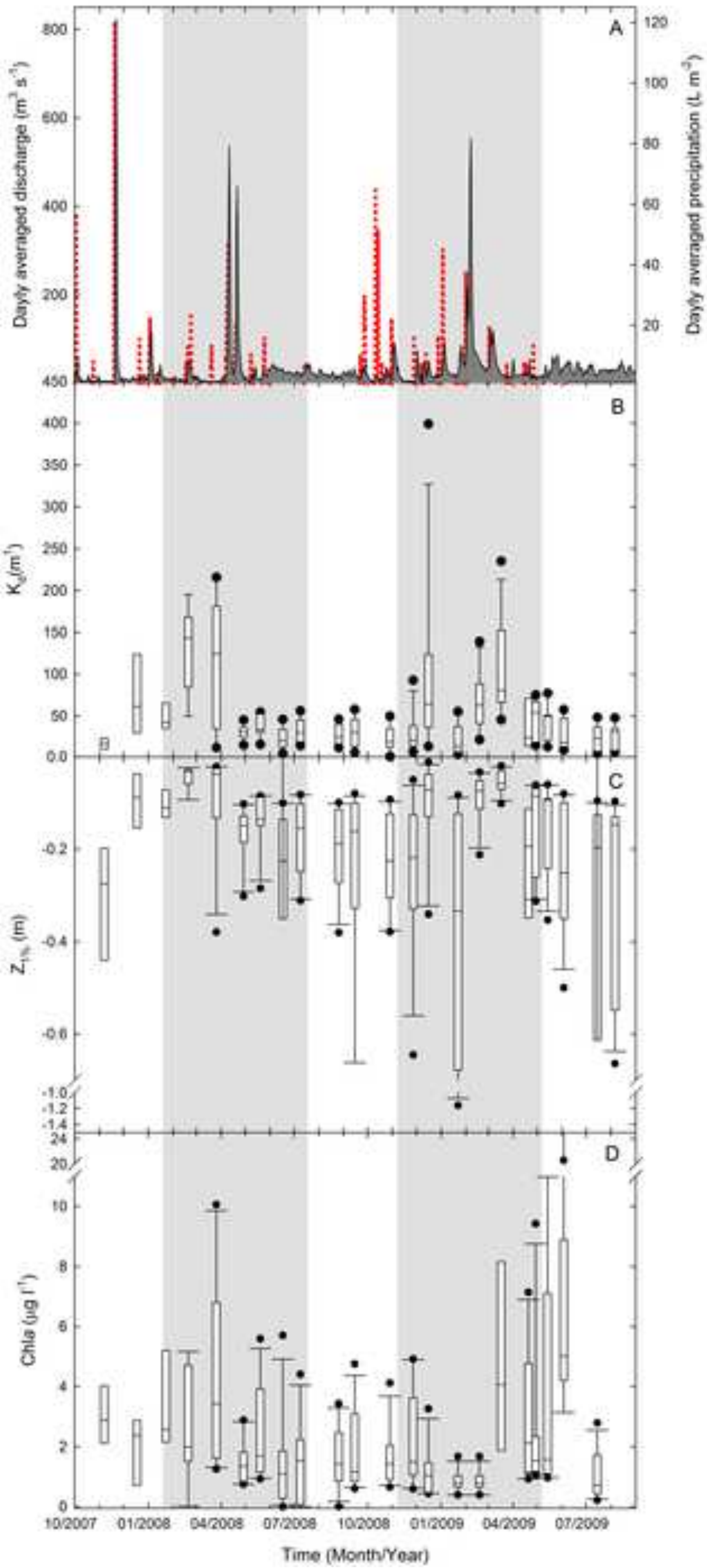

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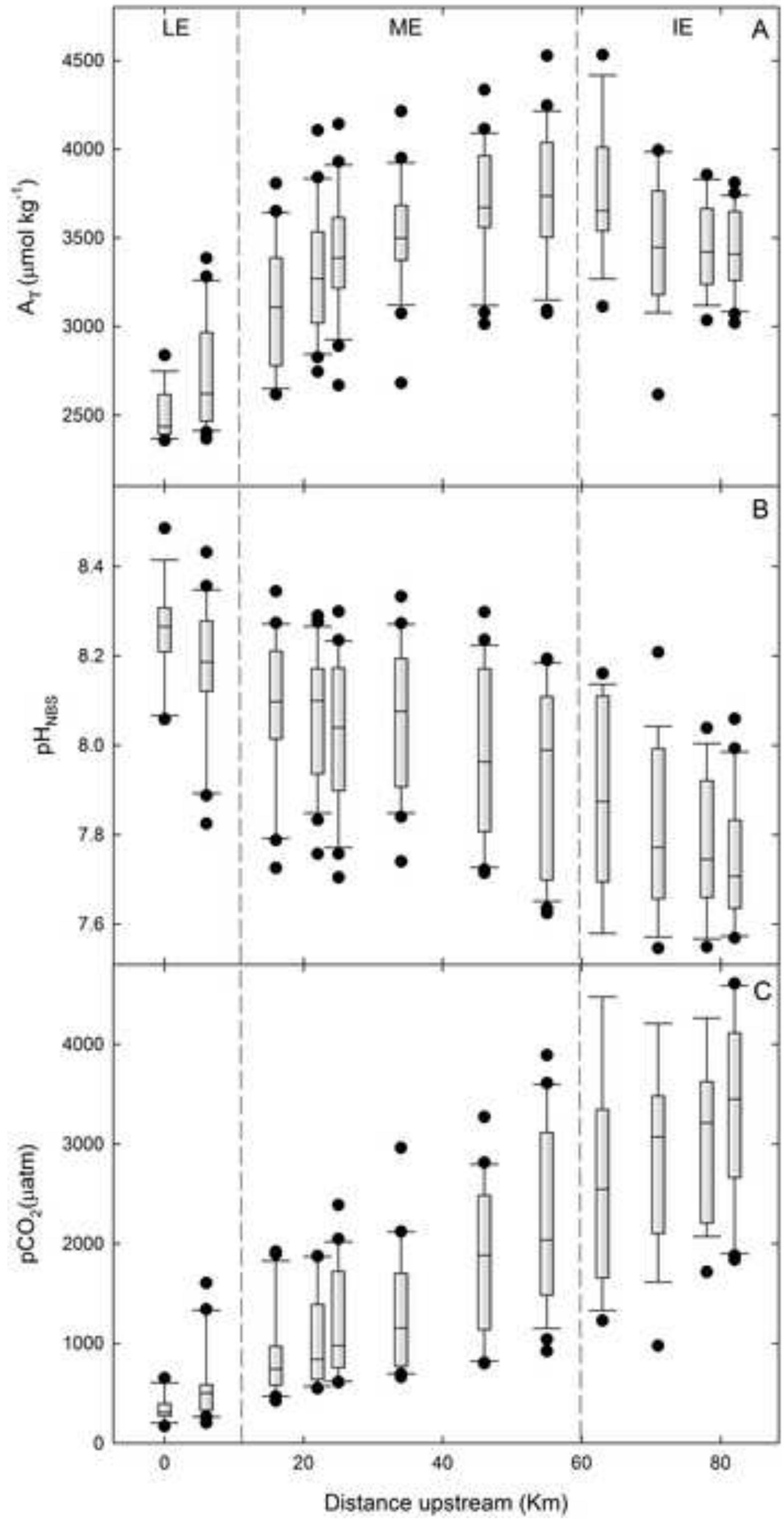

Figure6

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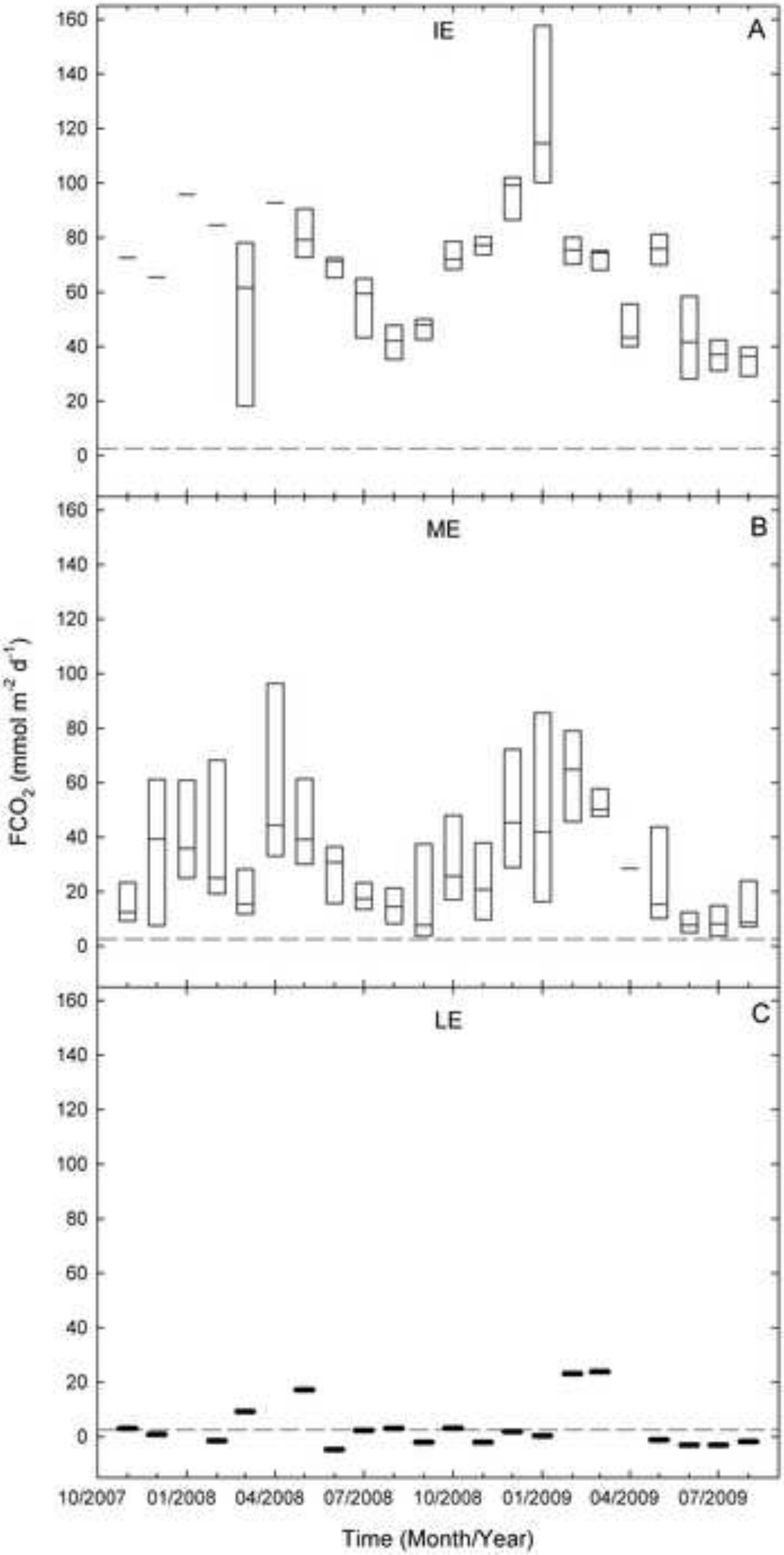

Figure7
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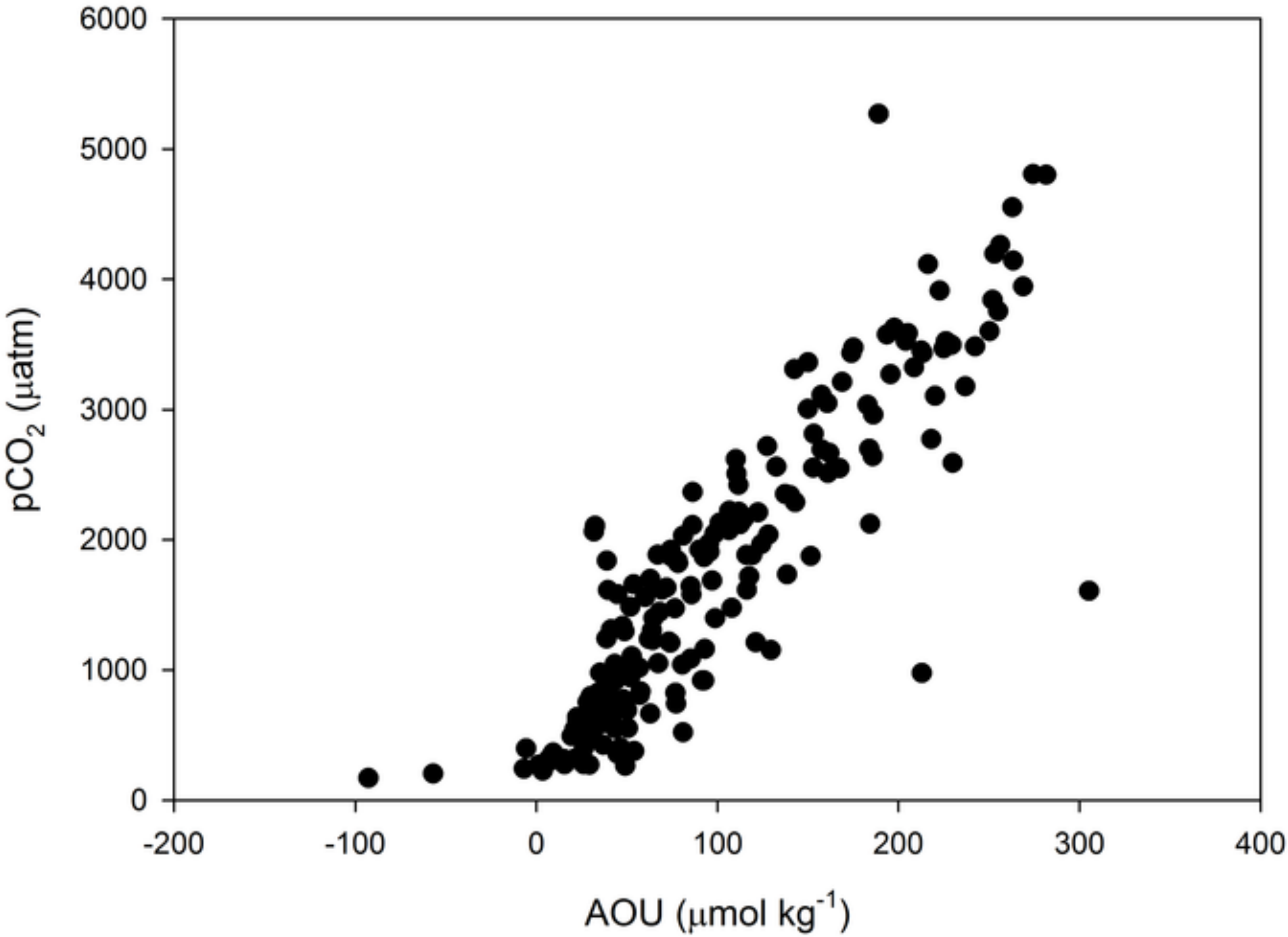

Figure8

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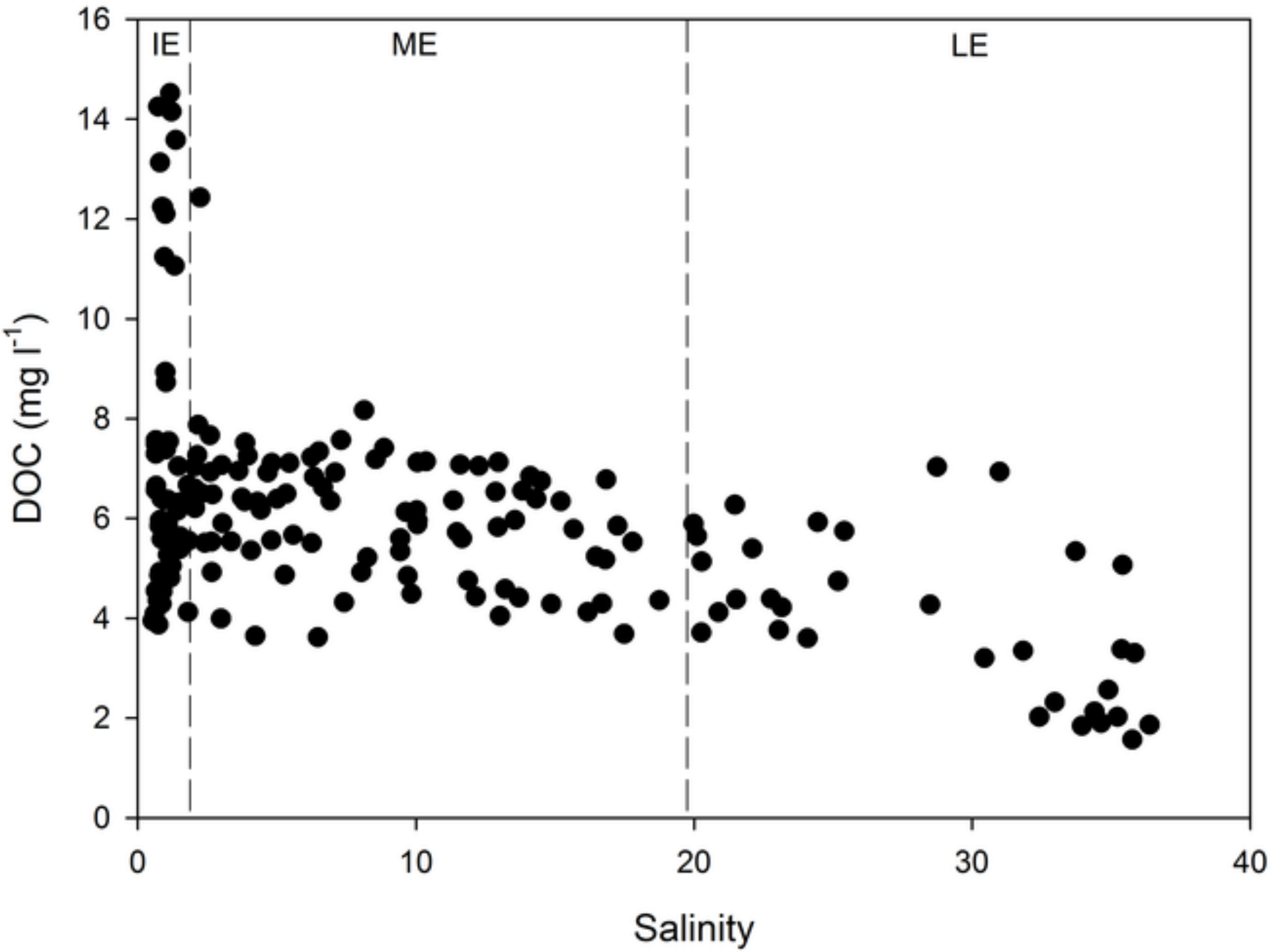

Figure9

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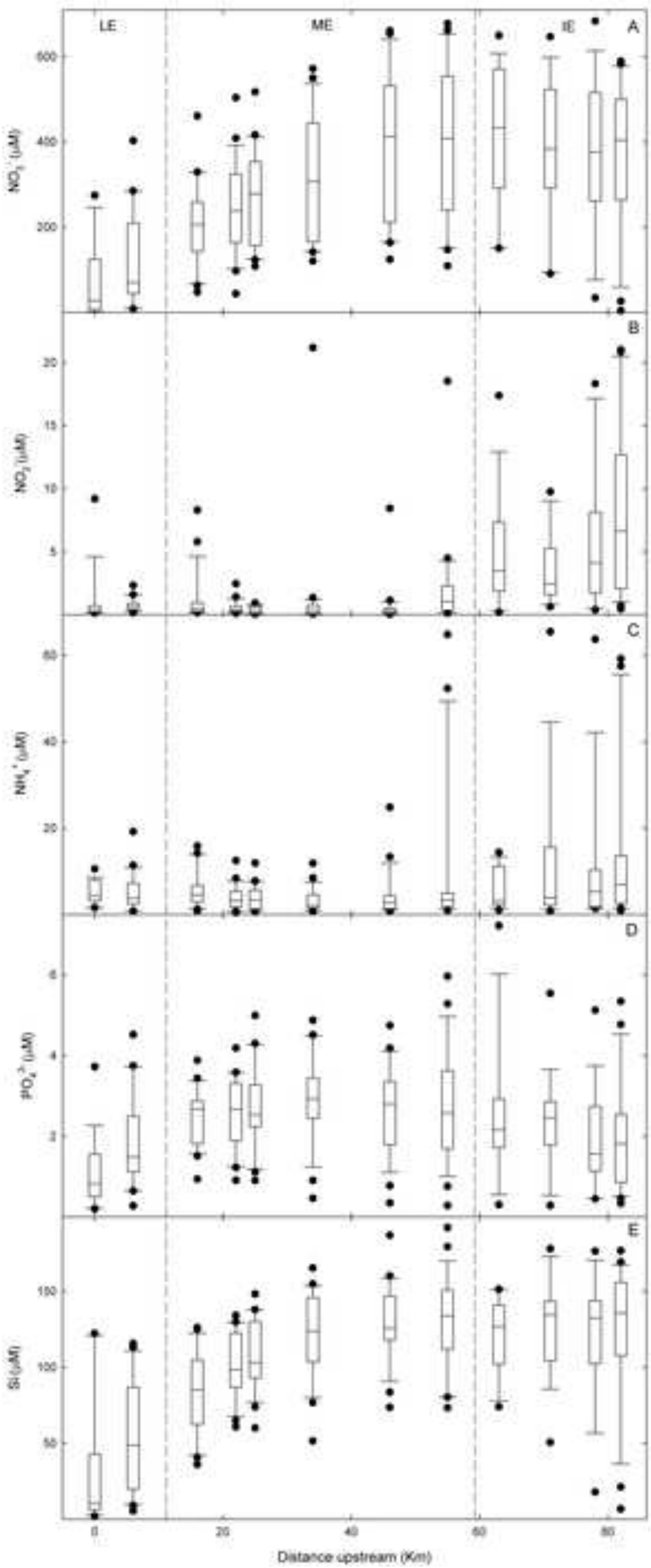

Table1

	Lower estuary	**Middle estuary**	**Inner estuary**
Area (km^2)	40.1	23.8	7.2
Temperature (ºC)	18.5±4.6	18.2±5.3	19.3±5.8
Salinity	30.0±6.4	9.2±6.8	1.1±0.6
Turbidity (FNU)	111±376	769±1116	310±271
DO (mg l^{-1})	6.6±1.0	6.4±1.3	4.0±1.6
pH_{NBS}	8.2±0.1	8.0±0.2	7.8±0.2
A_T (µmol kg^{-1})	2633±273	3468±403	3505±328
DIC (µmol kg^{-1})	2613±285	3442±389	3523±300
pCO_2 (ppm)	477±319	1440±807	3009±958
PO_4^{3-} (µM)	1.5±1.1	2.7±1.0	2.2±1.4
Si (µM)	42.5±38.0	112.5±30.3	122.7±36.3
NO_3^- (µM)	99.0±103.3	302.8±154.9	390.3±162.4
NO_2^- (µM)	0.9±1.5	1.1±2.6	5.9±5.4
NH_4^+ (µM)	5.3±3.6	5.2±8.3	19.1±49.4
DOC (mg l^{-1})	3.8±1.7	6.0±1.6	6.9 ±2.8
DON (mg l^{-1})	15.1±21.7	61.8±56.8	92.6±90.6
Chl*a* (µg l^{-1})	2.9±3.2	1.8 ±1.4	3.3±3.5
SPM(mg l^{-1})	771±504	779±790	360±372
PIM (mg l^{-1})	667±473	679±708	310±355
POM (mg l^{-1})	104±44	100±199	50±43
Kd (m^{-1})	55.9±38.0	57.9±57.5	26.6±27.5

Table2

	Winter	Spring	Summer	Autumn
pH_{NBS}	7.9±0.1	8.1±0.2	8.1±0.1	8.0±0.1
A_T (μmol kg^{-1})	3256±513	3114±658	3185±590	3280±572
DIC (μmol kg^{-1})	3291±520	3095±661	3120±561	3290±584
pCO_2 (μatm)	2063±862	1489±651	1216±387	1924±780

Table3

Zone	Winter flux (mmol C m^{-2} d^{-1})	Spring flux (mmol C m^{-2} d^{-1})	Summer flux (mmol C m^{-2} d^{-1})	Autumn flux (mmol C m^{-2} d^{-1})	Total (mmol C m^{-2} d^{-1})	Annual flux (mol C m^{-2} yr^{-1})
LE	12.6±18.7	-0.7±6.9	0.6±3.0	1.0±3.8	3.4±8.1	1.2±0.6
ME	44.5±25.7	28.1±23.8	15.0±10.6	30.2±21.2	29.4±20.3	10.7±1.1
IE	83.9±31.0	60.2±21.0	43.4±10.5	80.2±12.0	66.9±18.6	24.4±1.7
TOTAL	47.0±35.7	29.2±30.5	19.7±21.7	37.1±40.0	99.7±31.9	36.4±11.7